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GROWTH RESPONSES TO ORGANIC COMPOUNDS BY TOBACCO SEEDLINGS IN ASEPTIC CULTURE[1]

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INTRODUCTION

Investigations on the responses of plants to organic compounds of natural and synthetic origin have recently tended to assume considerable importance. The increased knowledge of the biochemistry of plants thus obtained has been utilized for making more practical the methods of producing mutants, rooting cuttings, and preventing fruit drop and for improving materials for the protection of plants against micro-organisms and insects. On the other hand, soil toxicity due to organic substances has been shown to exist (*7*).[2] The action of crop residues on the growth of subsequent crops (*3*) also may be partially of similar origin. Knudson (*4*) and others demonstrated that sugars and other compounds may be absorbed by the plant through its roots and lead to increased growth. No necessity exists, therefore, to justify the further use of this experimental procedure.

The studies dealt with in this paper originated when the observation was made that seedlings of the Robinson strain of Maryland Medium Broadleaf tobacco grown in aseptic culture under low-intensity artificial light at constant temperature did not pass through the rosette stage on an 8-hour day. The attempt to eliminate this formative effect of light led to a study of the influence of organic compounds of natural origin on growth. Though most of these data are for tobacco seedlings grown with a short day, it is believed that they do not differ in any important degree from those obtainable for plants grown with a long day.

MATERIALS AND METHODS

Seedlings of Maryland Medium Broadleaf tobacco were grown aseptically in 200-cc. Pyrex Erlenmeyer flasks containing 50 cc. of nutrient solution solidified with agar. The solution used was that developed by McMurtrey (*6*), and the 20 × solution contained water, 1,000 cc.; $Ca(NO_3)_2.4H_2O$, 29.16 gm.; KNO_3, 2.19 gm.; $Mg(NO_3).6H_2O$, 3.19 gm.; KH_2PO_4, 5.75 gm.; $MgSO_4.7H_2O$, 3.08 gm.; and NH_4Cl, 1.50 gm. The micronutrients in this concentrated solution

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[2] Italic numbers in parentheses refer to Literature Cited, p. 91.

comprised zinc, 10.0 mg.; copper, 2.5 mg.; manganese, 20.0 mg.; and boron, 10.0 mg. Iron was added separately to the diluted solution as ferric chloride at a concentration of 3 p. p. m. After dilution of this solution and addition of iron, 2 percent of sucrose, and 1.15 percent of agar, the medium was heated and put in flasks, which were then plugged with absorbent cotton and sterilized at 15 pounds' pressure for 30 minutes. The organic compounds under test were added to the flasks before they were filled with the medium.

Seed was sterilized with 1:1,000 silver nitrate for 15 minutes, washed with three changes of distilled water, and poured into previously sterilized petri dishes containing several layers of filter paper. The seedlings were transferred to the flasks after a germination period of 6 to 7 days at 25° C. The use of a pair of magnifying spectacles (2.5 ×) was helpful. A flamed platinum hook with a sharpened 2-mm. prong was moistened with sterile distilled water and brought in contact with the seedling, and the latter was transferred to the agar. The seedling adhered first to the platinum needle and then to the agar. Seedlings with a total length of 5 mm. were readily handled, as the radicles could be pushed into the agar with the back of the prong.

The seedlings were grown at 25° C. in lighted incubators under 3,500° white fluorescent lamps furnishing 500 foot-candles of illumination for 28 days. On harvesting, the seedlings were freed from the agar, rinsed with water, pressed between absorbent paper, and dried at 103° to 105° for 4 hours. Usually the values shown are the average of four duplicate cultures. The cultures were weighed in pairs. Contaminated cultures and abnormal plants were discarded. Injury to seedlings during transfer, immersion of cotyledons, and exposure of the radicles caused marked reductions in growth. Rejections due to these causes were about 10 percent.

EFFECTS OF SUGARS ON GROWTH

Growth in a medium containing sugars under the experimental conditions used is shown in table 1. The average dry weight obtained after 28 days of growth with 8 hours of light daily was 4 mg. The seedlings formed stems immediately and four to six leaves of good color. With continuous illumination the total yield per plant averaged 68.9 mg., the leaves were normal in shape and color, and the rosette stage was just on the point of termination. The main effect of sucrose (2 percent) was on the vigor and rate of growth of the seedling; the yields became 19.7 mg. with 8 hours of daily illumination and 150.5 mg. with continuous illumination.

The effects of other sugars and compounds listed in the table were studied on seedlings grown under 8 hours of daily illumination. Only with D-glucose and D-fructose did growth increase approach that with sucrose. D-fructose, however, caused root injury. None of the other compounds tested, with the possible exception of D-xylose, caused an increase in growth.

TABLE 1.—*Growth of tobacco seedlings, Maryland Medium Broadleaf, with various sugars in aseptic culture for 28 days at 25° C. and 500 foot-candles of fluorescent white light for 8 hours daily*

Treatment or compound (20 gm. per liter)	Average total yield	Appearance [1]	Treatment or compound (20 gm. per liter)	Average total yield	Appearance [1]
	Milligrams			*Milligrams*	
Control:			L-Arabinose	1.4	S, 9
8-hour day	4.0	S, 8	D-Mannitol	2.3	S, 8
8-hour day with sucrose	19.7	S, 8	Calcium citrate	3.6	S, 8
Continuous day	68.9	R, 10	Calcium D-gluconate	3.9	S, 6
Continuous day with sucrose	150.5	R, 10	Calcium 2-keto-D-gluconate	.9	S, 4
D-Glucose	15.6	S, 8	Calcium 5-keto-D-gluconate	.5	S, 4
D-Fructose	14.6	[2] S, 8	Calcium D-lactate	.7	S, 4
D-Mannose	1.6	R, 8	Potato starch	1.1	S, 6
D-Galactose	0	–, 0	Sucrose+D-mannose [3]	18.9	[2] R/2, 8
L-Sorbose	.8	S, 8	Do.[4]	11.9	[2] R, 10
D-Xylose	7.5	S, 8	Do.[5]	6.3	[2] R, 10
D-Arabinose	0	–, 4			

[1] S, Shoot or stem; R, rosette; R/2, partial rosette. Rated from 0 (white) to 10 (dark green).
[2] Short roots, injury.
[3] Mannose at 10 gm. per liter.
[4] Mannose at 20 gm. per liter.
[5] Mannose at 30 gm. per liter.

D-mannose appeared to be the only compound that inhibited stem formation, that is, aided passage through the rosette stage. It was not possible to eliminate premature stem formation with D-mannose, however, without decreasing yield. A mixture of 10 gm. of D-mannose and 20 gm. of sucrose was partially effective in promoting rosette formation without seriously diminishing yield.

Figure 1 illustrates the types of growth obtained on an 8-hour and a 24-hour day with and without sucrose. Growth with various sugars is shown in figure 2.

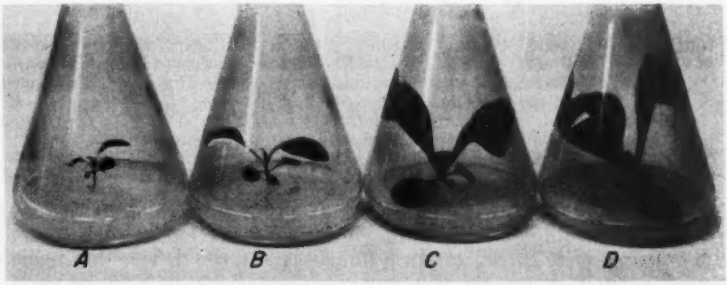

FIGURE 1.—Maryland Medium Broadleaf tobacco seedlings, 3 weeks old, grown aseptically on mineral agar at 25° C. with 500 foot-candles of fluorescent white light: *A*, With 8 hours of daily illumination but without sugar in medium; *B*, with 8 hours of daily illumination and 2 percent of sucrose in medium; *C*, with continuous illumination but without sucrose in medium; *D*, with continuous illumination and sucrose in medium.

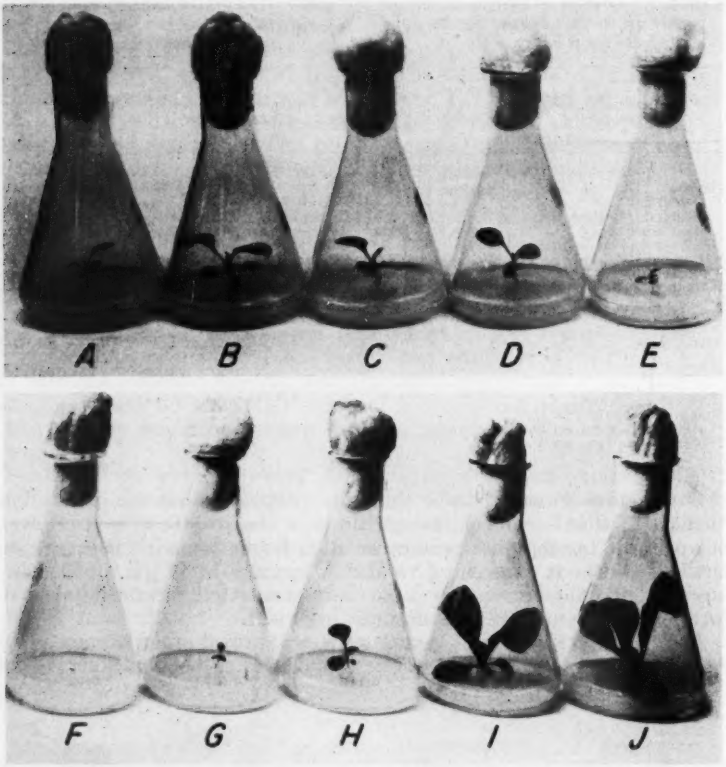

FIGURE 2.—Maryland Medium Broadleaf tobacco seedlings, 3 weeks old, grown aseptically on mineral agar at 25° C. with 500 foot-candles of fluorescent white light: *A* to *H*, With 8 hours of daily illumination—without sugar (*A*) and with 2 percent each of sucrose (*B*), D-glucose (*C*), D-fructose (*D*), D-mannose (*E*), D-galactose (*F*), L-sorbose (*G*), and D-xylose (*H*); *I* and *J*, with continuous illumination—without sucrose (*I*) and with 2 percent of sucrose (*J*).

EFFECTS OF AMINO ACIDS ON GROWTH

The results of tests with amino acids on growth of tobacco seedlings are shown in table 2. All media contained 2 percent of sucrose, and the cultures were grown on an 8-hour day. Growth was diminished almost without exception at a concentration of 200 p. p. m. of amino acid. *d*-Arginine hydrochloride, *l*-cystine, and *dl*-ornithine hydrochloride proved least toxic in the order given.

Toxicity at a level of 200 p. p. m. was associated with excessive acidity in the case of *l*-aspartic acid (pH=3.98), *d*-glutamic acid (pH=4.08), *l*-histidine dihydrochloride (pH=4.07), and *d*-lysine dihydrochloride (pH=3.99). Cultures of excessive acidity were easily recognized by failure of the agar to gel. The acidity factor, however, was probably of minor importance in itself at the above-mentioned acidities. Glycine (pH=4.96), *l*-hydroxyproline (pH=

TABLE 2.—*Growth of tobacco seedlings, Maryland Medium Broadleaf, with 2 percent of sucrose plus various amino acids in aseptic culture for 28 days at 25° C. and 500 foot-candles of fluorescent white light for 8 hours daily*

Control or compound (200 mg. per liter)	Average total yield	Appearance [1]	Control or compound (200 mg. per liter)	Average total yield	Appearance [1]
	Milligrams			*Milligrams*	
Control	20.1	S, 8	*dl*-Isoleucine [2]	1.8	R, 4
dl-Alanine	.4	------	*d*-Lysine dihydrochloride	2.0	R, 4
dl-α-Amino-*n*-butyric acid	0	------	*dl*-Methionine	4.8	S, 8
d-Arginine hydrochloride	18.3	S, 8	*dl*-Norleucine	.5	R, 2
l-Asparagine	.5	R, 0	*dl*-Ornithine hydrochloride	13.9	S, 8
l-Aspartic acid	2.0	R, 6	*dl*-β-Phenylalanine	10.1	S, 8
l-Cystine	15.6	S, 8	*l*-Proline	.1	R, 2
d-Glutamic acid	.6	R, 2	*dl*-Serine	0	------
Glutathione	1.4	R, 2	*dl*-Threonine	.2	R, 2
Glycine	.5	R, 1	*l*-Tryptophane	.6	R, 2
l-Histidine dihydrochloride	4.8	R, 8	*l*-Tyrosine	10.4	S, 8
l-Hydroxyproline	0	—, 0	*dl*-Valine	.3	R, 2
l-Leucine	9.2	S, 8			

[1] S, Shoot or stem; R, rosette. Rated from 0 (white) to 10 (dark green).
[2] Seedlings grown with *dl*-isoleucine showed inhibition in growth of apical bud, suckering, and strap leaves; all typical symptoms of frenching.

4.92), *dl*-isoleucine (pH=4.89), *l*-proline (pH=4.76), *dl*-serine (pH=4.78), *dl*-threonine (pH=4.78), and *l*-tryptophane (pH=5.14) were very toxic though having acidities comparable to that of the control (pH=4.51). All these acidities were measured at the time of harvest. Initial acidity of the control was equivalent to a pH of 5.11.

In no instance was shoot development inhibited by an amino acid at a concentration of 200 p. p. m. except when it checked all growth. Chlorosis when present was not distinctive as a rule but consisted in a uniform bleaching of all leaves except the cotyledons.

Marked and characteristic abnormalities occurred only in the presence of *dl*-isoleucine (*9*). The rosette condition persisted with *dl*-isoleucine because of inhibition of stem and sucker growth and profuse development of the axillary leaves. As compared with the controls, an excessive number of leaves were formed which exhibited a slight mottling and varied from narrow to "strap leaf." The general appearance of these leaves was very similar to that of leaves of tobacco plants affected with the disease known as frenching. Divergencies, of course, existed. These differences consisted in the absence of a distinctive reticular chlorosis, the presence of curved leaf margins, the absence of pale bud and leaf margins, and the presence of the characteristic waving and ruffling of the strap-leaf margins.

The appearance of seedlings grown in a medium containing *dl*-isoleucine is shown in figures 3 and 4. Strap-leaf formation was noticeable in 14 days and occurred in the first pair of leaves formed. It is improbable that these responses were associated with an impurity, for similar results were obtained with two different samples purchased several years apart from the same manufacturer and with a third sample from a second manufacturer.

Additional tests were made with the most toxic of the amino acids at a lower level of concentration (50 p. p. m.). The results are given in table 3. Most of these amino acids were rather toxic under the conditions of the tests even at these high dilutions.

TABLE 3.—*Growth of tobacco seedlings, Maryland Medium Broadleaf, with 2 percent of sucrose plus amino acids of high toxicity in aseptic culture for 28 days at 25° C. and 500 foot-candles of fluorescent white light for 8 hours daily*

Control or compound (50 mg. per liter)	Average total yield	Appearance [1]	Control or compound (50 mg. per liter)	Average total yield	Appearance [1]
	Milligrams			*Milligrams*	
Control	17.9	S, 8	*dl*-Isoleucine [2]	3.1	[4] R, 4
dl-Alanine	.2	R, 2	Do. [5]	1.7	[6] R, 2
l-Aspartic acid	9.6	S, 6	*d*-Lysine dihydrochloride	11.0	S, 6
d-Glutamic acid	.7	R, 2	*l*-Proline	.4	S, 2
Glycine	.4	R, 2	*dl*-Serine	.3	S, 2
l-Histidine dihydrochloride	11.2	S, 6	*dl*-Threonine	1.6	S, 1
l-Hydroxyproline	.1		*l*-Tryptophane	8.8	R, 8
dl-Isoleucine	4.2	[3] R, 6	*dl*-Valine	.5	R, 1

[1] S, Shoot or stem; R, rosette. Rated from 0 (white) to 10 (dark green).
[2] Narrow leaves.
[3] 100 mg. per liter.
[4] Narrow and strap leaves.
[5] 200 mg. per liter.
[6] Strap leaves.

The frenching response with *dl*-isoleucine persisted but with diminished severity. For example, strap leaves were not formed at the lowest concentration though the widths of the leaves were decreased by more than half. It is possible, however, that a search would reveal conditions under which the action of *dl*-isoleucine would be exhibited at even a lower concentration than 50 p. p. m. It was the

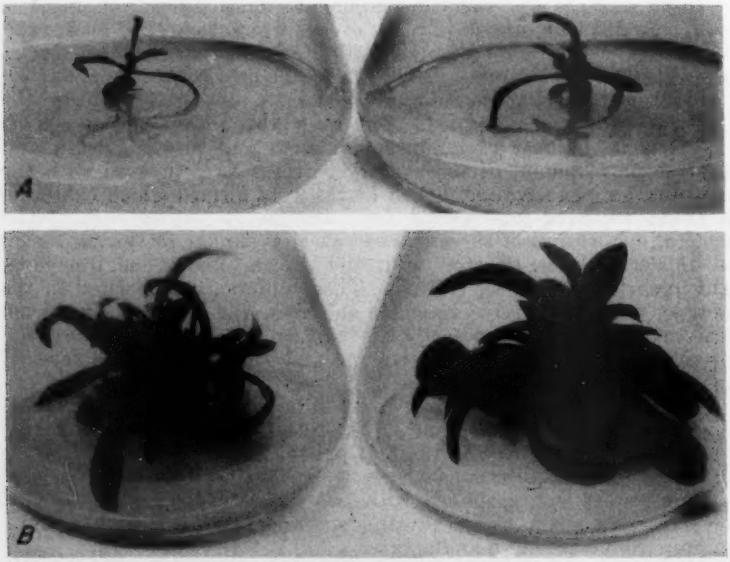

FIGURE 3.—Maryland Medium Broadleaf tobacco seedlings grown aseptically at 25° C. with 500 foot-candles of fluorescent white light on mineral agar containing 2 percent of sucrose and 200 p. p. m. of *dl*-isoleucine: *A*, Seedlings 4 weeks old, grown with 8 hours daily illumination; *B*, same seedlings after being grown an additional month with continuous illumination.

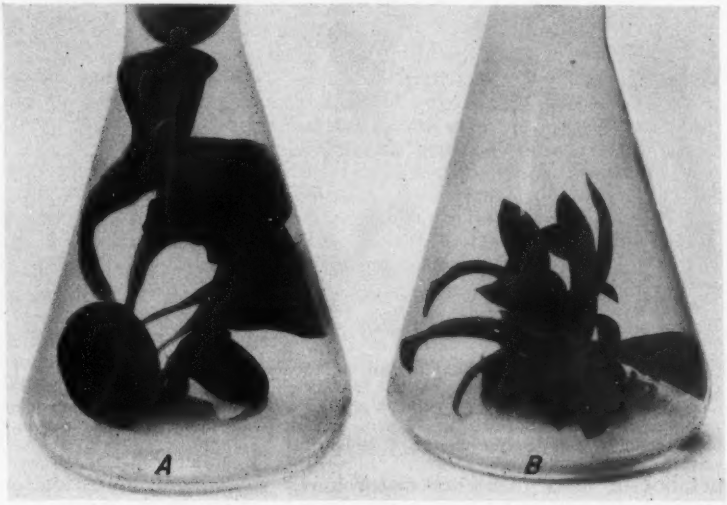

FIGURE 4.—Maryland Medium Broadleaf tobacco seedlings grown from September 5 to October 25, 1945, at 25° C. with 500 foot-candles of fluorescent white light and continuous illumination on mineral agar containing (*A*) 2 percent of sucrose and (*B*) 2 percent of sucrose plus 200 p. p. m. of *dl*-isoleucine.

only one of the 23 amino acids capable of causing a specific response of this nature at this concentration.

Other amino acids can modify the action of *dl*-isoleucine on growth of tobacco seedlings (fig. 5). When 50 mg. per liter of *dl*-isoleucine was used with 20 mg. of another amino acid, a wide difference in response appeared. Increases in growth were effected by *l*-aspartic acid, *l*-cystine, glycine, *l*-histidine dihydrochloride, *l*-leucine, *d*-lysine dihydrochloride, *dl*-methionine, *dl*-norleucine, *dl*-β-phenylalanine, *l*-proline, and *dl*-threonine. The controls with *dl*-isoleucine had narrow leaves showing a diffuse chlorosis, whereas all admixtures except those with *dl*-alanine, *l*-hydroxyproline, and *l*-tryptophane produced seedlings with leaves having the clearly defined reticular chlorosis so characteristic of frenching. *dl*-Alanine, *l*-hydroxyproline, and *l*-tryptophane caused sharp decreases in yield. *dl*-Alanine and *l*-tyrosine, however, seemed to neutralize to some degree the abnormal morphological effects of *dl*-isoleucine, while *l*-hydroxyproline and *l*-tryptophane seemed to accentuate these (strap-leaf formation).

A summary of the characteristic symptoms of the various amino acid toxicities appears in table 4. The divergencies and similarities in gross morphology resulting from excesses of individual natural amino acids are listed. The quantities involved ranged from 0.25 to 2.5 mg. per seedling, if only those acids included in the table are considered. Symptoms of abnormality vary greatly and include loss of dominance, or death of the apical bud (retention of the rosette condition and inhibition of stem growth and of suckering); various leaf mottlings, streaks, and blotches; and narrow, strap, ruffled, twinned, and rim-bound leaves. Therefore, abnormalities in protein

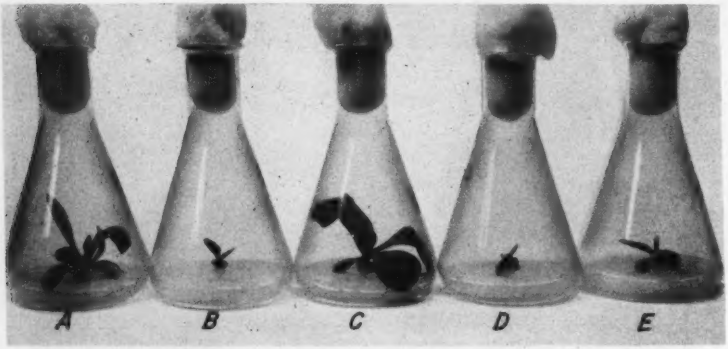

FIGURE 5.—Maryland Medium Broadleaf tobacco seedlings grown at 25° C. with 500 foot-candles of fluorescent white light and continuous illumination on mineral agar containing 2 percent of sucrose and 50 p. p. m. of *dl*-isoleucine plus additional amino acid at 20 p. p. m.: *A*, None (control); *B*, *dl*-alanine; *C*, glycine; *D*, *l*-hydroxyproline; *E*, *dl*-isoleucine.

metabolism consequent to disturbances of mineral nutrition or physical-environmental factors may comprise in part the mechanism through which such aberrations are made evident as abnormalities in gross morphology.

TABLE 4.—*Symptoms in tobacco seedlings, Maryland Medium Broadleaf, due to excessive concentrations of the more toxic natural amino acids*

Compound	Concentration	Symptoms of toxicity other than retarded growth
	P. p. m.	
dl-Alanine	5	Chlorotic spots at apex and margins.
l-Aspartic acid	50	Large, faintly chlorotic spots.
d-Glutamic acid	20	Large white blotches.
Glycine	40	Faint interveinal chlorosis.
l-Histidine dihydrochloride	50	Small, faintly chlorotic spots.
l-Hydroxyproline	5	Inhibition of buds; streak chlorosis; thick, down-curled leaves; brown, dead leaves; rim-bound leaves.
dl-Isoleucine	50	Loss of apical dominance, narrow leaves, mottling.
d-Lysine dihydrochloride	50	Reticular chlorosis.
dl-Norleucine	20	Inhibition of secondary root growth.
l-Proline	40	Faint interveinal chlorosis.
dl-Serine	40	General mottling.
dl-Threonine	50	White leaves.[1]
l-Tryptophane	20	Loss of apical dominance; isolated ruffled strap leaves; rim-bound, twinned, pointed, and withered-zone leaves; suckering.
dl-Valine	20	Large white blotches.

[1] White leaves are believed to be a general symptom due to excess quantities of amino acids; 20 p. p. m. of the amino acids listed as causing this condition did not prevent normal morphological growth. Specific abnormalities in gross morphology will probably be found to occur at intermediate concentrations. An interesting symptom, root blackening, was also caused by high concentrations of tyrosine.

These growth effects of the free, natural amino acids, like those of D-mannose and commercial peptones (p. 89), would seem to minimize the need for assuming the existence of hormones regulating all the activities of plants, because it is evident that the ordinary metabolic products of the cell in themselves can exert a hormone action on the growth processes of plant tissues. The hormone action of other natural metabolites also may be the explanation for the similarities in response to dissimilar synthetic growth substances causing root initiation, epinasty, callus formation, and other growth correlations.

EFFECTS OF VITAMINS AND PEPTONES ON GROWTH

None of the vitamins used (table 5) appeared more than very slightly beneficial to the growth of Maryland Medium Broadleaf tobacco. Pyridoxine and ascorbic acid gave slight increases in yield which, however, did not exceed the limits of experimental precision.

It is interesting to note that certain peptones also brought about slight increases in yield. The important feature about these responses, however, was not the slight increases in yield that were obtained, but the evidence for the relative nontoxicity of peptones and proteoses as compared with amino acids and the peptide glutathione. Some of these peptones (Bacto-tryptose, Bacto-protone, Proteose-peptone) appeared favorable to retention of the rosette condition despite the short day, but the response did not appear regularly.

TABLE 5.—*Growth of tobacco seedlings, Maryland Medium Broadleaf, with 2 percent of sucrose plus vitamins or peptones in aseptic culture for 28 days at 25° C. and 500 foot-candles of fluorescent white light for 8 hours daily*

Control or compound (1.0 mg. per liter)	Average total yield	Appearance [1]	Control or compound (200 mg. per liter)	Average total yield	Appearance [1]
	Milligrams			*Milligrams*	
Control	19.6	S, 8	Casamino acids [2]	6.5	S, 2
Biotin [3]	20.4	S, 8	Bacto-peptone [2]	22.7	S, 8
Thiamine chloride	14.8	S, 8	Peptone (Witte)	20.4	S, 8
Pyridoxine	23.8	S, 8	Malt extract [2]	18.8	S, 8
Nicotinic acid	19.5	S, 8	Yeast extract [2]	1.8	S, 2
Riboflavin [4]	19.0	S, 8	Bacto-tryptose [2]	22.8	R/2, 8
Ascorbic acid	22.4	S, 8	Bacto-protone [2]	18.0	R/2, 8
Calcium *d*-pantothenate	19.7	S, 8	Bacto-tryptone [2]	16.9	S, 8
p-Amino benzoic acid	19.8	S, 8	Proteose-peptone No. 4 [2]	23.1	R/2, 8
Folic acid [5]	19.3	S, 8	Neopeptone [2]	19.3	S, 8
Indole-3-propionic acid	11.7	S, 8			
Indole-3-acetic acid	11.1	S, 8			

[1] S, Shoot or stem; R/2, partial rosette. Rated from 0 (white) to 10 (dark green).
[2] From Difco Laboratories.
[3] 10 gammas per liter.
[4] 0.4 mg. per liter.
[5] 0.1 mg. per liter. Courtesy of Prof. Roger J. Williams.

EFFECTS OF PURINES AND PYRIMIDINES ON GROWTH

Excesses of metabolites other than amino acids may behave similarly in producing abnormalities in gross morphology. At concentrations of 200 p. p. m., for example, adenylic acid, guanylic acid, uracil, guanine, guanosine, and hypoxanthine brought about faint mottling. Adenine caused formation of white leaves because of excessive toxicity. Faint, glossy, dark-green leaf blotches also were evident with xanthine, guanosine, and hypoxanthine. With all of these products of nucleic acid decomposition except adenine yields were almost normal. Growth with adenylic acid, uracil, and xanthine was, indeed, slightly better than that of the controls. Glutathione (table 2), a tripeptide, was also rather toxic and would probably cause production of specific symptoms at its toxic threshold.

DISCUSSION

Persistence of the rosette stage preliminary to shoot elongation varies considerably with different varieties of tobacco. The variety used in these experiments is characterized by a well-defined preliminary rosette stage. The day-length experiments of Allard and Garner (*1*) would indicate that duration of the rosette condition is briefer with long daily periods of solar illumination than with short ones. With the environmental conditions employed in these experiments this response appeared to be reversed. The plants grown with a short day began stem elongation almost immediately, whereas those in continuous illumination remained in the rosette stage for a month or more. The factor responsible was probably low light intensity, but high moisture and relative humidity may also have contributed to the reversal in response. Reversal is viewed primarily, however, as a low-nutrition response.

It is probably unnecessary to assume the presence of a rosette-forming hormone in this reaction of tobacco. The data seem to indicate that variations in the proportions and concentrations of ordinary biochemical constituents of the plant may bring about modifications in duration of the rosette stage. The difficulty in demonstrating this interpretation experimentally lies in proving that the components of the chemical mixture used to cause reversion to rosette formation are actually those so functioning in the plant. Biochemical data are needed.

The characteristic and specific response of tobacco seedlings to *dl*-isoleucine is of great interest, particularly if it proves to be the primary cause of frenching in tobacco.[3] This disease is of world-wide occurrence, but little is known of its cause. Response of tobacco to *dl*-isoleucine in aseptic culture is very similar to frenching in the field, considering the wide differences in these environments. Moreover, all 3 samples of this amino acid that were tried were equally effective and varied with the concentrations used. The ineffectiveness of about 60 other biochemical compounds to bring about a similar response would indicate the degree of specificity involved in this reaction. McMurtrey (*5*), however, was also able to duplicate the symptoms of frenching to some degree with thallium salts. Shear and Ussery (*8*) found that this tobacco disease occurred in the absence of thallium under field conditions.

The mechanism is unknown whereby specific characteristic symptoms in the green plant are brought about through mineral deficiencies and toxicities. Though an assumption of direct action might appear plausible for macronutrients, nutritive deficiencies of micronutrients at least could lead to characteristic syndromes only indirectly. The data presented on amino acids reveal the existence of a mechanism for the production of specific morphological abnormalities dependent upon irregularities in protein metabolism. Variations in formation and degradation of proteins under abnormal environments could lead to accumulation in excess of specific free amino and other acids,

[3] A similar response to *dl*-isoleucine is given by Xanthi Turkish tobacco in soil and solution culture in the greenhouse and by Connecticut Broadleaf in soil. Symptoms of mild frenching—bud inhibition, pale buds, narrow leaves with pale rims, and reticular chlorosis—were quite characteristic of the Connecticut Broadleaf variety in soil.

purines, pyrimidines, and peptides sufficient to produce a direct and harmful effect on growth and color of leaves, buds, and stems. The phenomena of amino acid toxicities have a range ample to account for at least some of the specific morphological symptoms encountered with disturbances in mineral nutrition. The validity of this interpretation is further supported by the data demonstrating the relative innocuousness of proteoses and peptones and by the modifications in symptoms resulting from amino acid admixture and from concentration.

Morphological abnormalities in plant growth are known to take place in darkness and are accompanied by an increase in free amino acids. Bennett (*2*) reported that iron chlorosis in spur leaves of pears is accompanied by an increase in soluble nitrogen.

SUMMARY

Seedlings of the Robinson strain of tobacco, Maryland Medium Broadleaf, were grown for 28 days in 200-cc. Pyrex Erlenmeyer flasks under aseptic conditions at 25° C. with 500 foot-candles of fluorescent white light. Each flask contained 50 cc. of a mineral-agar medium. Of about 60 sugars, amino acids, vitamins, and peptones added to the medium, only sucrose, D-glucose, D-fructose, and perhaps D-xylose were definitely beneficial to growth. Other compounds, particularly amino acids, were toxic at concentrations of 5 to 200 p. p. m. A specific toxicity response to *dl*-isoleucine duplicated most of the gross characteristics of the disease known as frenching, especially strap-leaf formation, reticular chlorosis, inhibition of all stem elongation, and large increase in leaf number. Admixtures of other amino acids with *dl*-isoleucine led to wide variations in response from diminution to accentuation of the symptoms of frenching. Individual acids caused distinctly different patterns of gross morphological abnormalities. It is suggested that excesses of free amino acids due to abnormalities in protein metabolism may contribute to the formation of characteristic patterns of symptoms in plants because of disturbances in mineral nutrition. Failure to pass through the rosette stage under short daily illuminations could be corrected partially by addition of D-mannose and certain peptones. Commercial peptones (peptones, proteoses) were relatively nontoxic as compared with the free amino acids.

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CONGENITAL MALFORMATIONS OF EYES OF SHEEP[1]

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INTRODUCTION

Maternal body temperature and moisture in mammals are sufficiently uniform to prevent these factors from interfering with normal development of the embryo. Lack of oxygen, excess of carbon dioxide, dietary deficiencies, and the presence of toxic substances, however, do interfere with and retard the rate of normal development.

The relation between a faulty maternal diet and anomalies in the offspring has been reported by many investigators. Hale (*5*)[2] and Moore, Huffman, and Duncan (*8*) found many congenital malformations in pigs and calves maintained on a vitamin-A-deficient diet. Warkany and his coworkers (*15*, *16*) described skeletal abnormalities in the offspring of rats due to maternal nutritional deficiencies. Franke and Tully (*4*) produced chick monsters by feeding grains which contained selenium, and Landauer (*6*) produced various types of abnormalities with organic selenium in creeper and normal strains of fowl. Beath et al. (*1*) reported malformations in the progeny of sheep which were grazed on seleniferous areas.

It is evident that selenium can interfere with the normal development of the embryo and that it can produce malformations in the progeny. The process by which selenium produces malformations was suggested by the investigations of Wright (*19*), when he demonstrated that selenium injures the oxidative mechanism of adult tissues. If selenium interferes with tissue respiration, those organs which have the highest growth rate during embryonic development, that is, the eyes, head, and extremities, would be the most seriously affected. This has been the case in all the malformations that the writers have observed in the lambs of sheep grazed on seleniferous areas.

Reports on selenium malformations up to the present time have dealt with the structural anomalies observed. The present paper presents observations on the gross and histological changes that occurred in the eyes of lambs from mothers which had grazed on seleniferous areas. The malformations involved the extremities and the eyes. This paper deals only with the gross and histological changes in the malformed eyes.

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[2] Italic numbers in parathenses refer to Literature Cited, p. 102.

MATERIAL AND METHODS

Two hundred and fifty malformed lambs were born in a group of 2,100 ewes grazed on a seleniferous area. Seventy-five percent of the malformed lambs died at birth and 10 percent died between the ages of 3 to 5 months. The lambs that lived longer were dwarfed, the extremities and eyes were deformed, and the reproductive organs were underdeveloped. Similar malformations were observed by Beath et al. (*1*) in animals on seleniferous areas in Wyoming.

The malformations of the eyes of 16 animals were studied. In order to observe the sequence of changes which occur during growth, the lambs were killed at ½, 1, 2, 3, 4, and 6 months of age. Analyses for selenium in the blood, liver, and kidney were carried out on all animals. In the younger group the selenium content of the liver ranged from 3.5 p. p. m. to 5 p. p. m. and in the older group from 0.8 to 1.5 p. p. m. In previous experiments the writers (*10*) had observed that about 30 percent of the daily selenium intake was eliminated in the urine provided no kidney injury was present. The tissues of newly born lambs, without congenital defects were free from or contained only traces of selenium.

All tissues were fixed in 10 percent formalin.

Studies of the various organs showed pathologic changes similar to those observed in the blind staggers type of selenium poisoning reported by Draize and Beath (*2*) and Rosenfeld and Beath (*12*).

GROSS CHANGES IN MALFORMED EYES

OBSERVATIONS

The eyes of 2- to 4-week-old lambs showed a single large transparent cyst protruding from the bulbar conjectiva. As the lambs matured these cysts ruptured and the eye proper could be observed. The normal position of the eye in the orbit was disturbed and the cornea was misplaced, either to the nasal or the temporal side. The cysts varied in size and were covered with fibrous tissue.

At necroscopy microphthalmia with multiple cysts and normal-size eyes without cornea and pupil were observed. The lenses were misplaced or absent and the whole organ appeared to be filled with clear, transparent fluid. The sclera was fused with the ethmoid, the frontal bones, or the floor of the orbit. In such cases the eyes were fixed and could not be rotated. Separation of the cysts from the orbit resulted in the rupture of the cysts (fig. 1, *A*). There were large pigmented epithelial and connective tissue folds overlapping the eyes (fig. 1, *B*). In the younger animals the eye appeared smaller (fig. 1, *C*) because of the smaller size of the cysts. In the older animals the only limiting factor imposed on the size of cysts was the space available within the orbit. The number of cysts varied from two to six per eye (fig. 1, *D*). The origin and insertions of the muscles of the eye could not be determined on account of the disorganized structural arrangement and the protrusion of the cysts. There were increased periorbital fat and some gelatinous embryonic tissue. The orbital cavity usually appeared normal, but occasionally some of the bones showed erosion and petechial hemorrhages caused by pressure of the cysts.

The eyes were sectioned through the anteroposterior axis. An eye of some sort was always present. No relation between the age of the

animals and the development of structures of the eyes could be observed. The increase in size was due to the enlargement of the cysts, not to development of the eyes. The malformations in the eyes of the same animal were not identical. Usually the deformities showed a more extreme manifestation in the right eye than in the left (fig. 2, A). Rawles ('9) observed that chick blastoderms of the head process on the left side have a higher developmental capacity than those on the right.

The various anomalies observed in the diffrent structurs of the eye may be described as follows:

Cornea.—The cornea was small and thickened and many times was

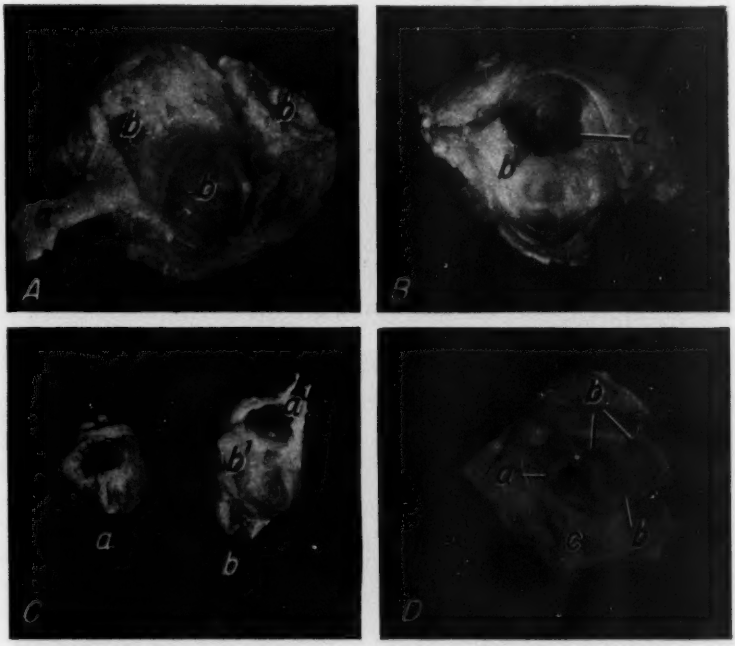

FIGURE 1.—Malformations of eyes of lambs from mothers that had grazed on seleniferous areas: *A*, *a*, Posterior view of eye, showing remnant of ruptured cyst which adhered to floor of orbit; *b*, posterior view of several large cysts. *B*, *a*, Pigmented epithelial fold covering anterior part of eye; *b*, large cyst with thin-walled elevated cysts; *C*, *a*, Eye of 2-week-old lamb showing microphthalmia; *b*, eye of 2-week-old lamb showing remnants of bulbar conjunctival cyst (*a'*) and posterior large cyst (*b'*). *D*, *a*, Misplaced eye; *b*, three small cysts; *c*, one large cyst. All X 0.7.

displaced laterally (fig. 2, *A*). A gray opacity obscured the normal division between the sclera and cornea. There was an extension of the episcleral tissue, and occasionally it showed some pigmentation.

Anterior and posterior chambers.—In many cases the division into anterior and posterior chambers was not well defined or was completely absent. In other cases the anterior chamber was shallow and the lens pushed forward, almost touching the cornea.

Lens.—Congenital absence of the lens was observed, usually when the eyes were rudimentary in size (fig. 2, *B*). Attachment of the lens to the retina or iris was observed in two cases. In one the lens appeared dumbbell-shaped and was covered by a continuous lens capsule. The lens was located postero-laterally; one part was within the eye while the other part projected into the cystic cavity (fig. 3, *A*).

Iris.—In all cases the iris was rudimentary or absent. The muscles of the iris were absent. Occasionally the rudimentary iris was attached and extended over the cornea.

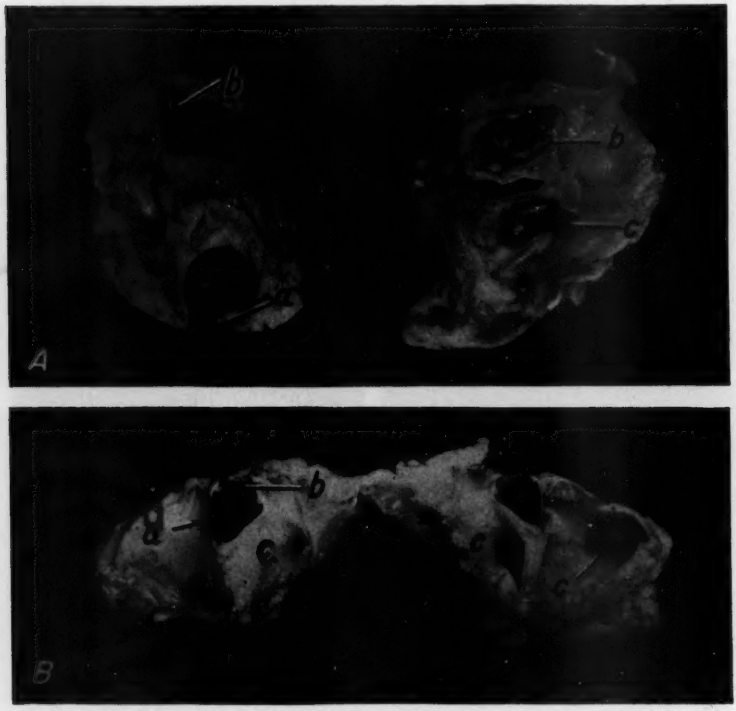

FIGURE 2.—*A*, Left eye shows more advanced development and higher structural differentiation than right eye: *a*, Cornea displaced laterally; *b*, cystic cavities and defects in walls of the eye; *c*, papillary projection on walls of cystic cavities. *B*, Microphthalmia rudimentary separation into anterior and posterior chambers; *a*, Eye filled with loose connective tissue, lens absent; *b*, displaced cornea; *c*, large cystic cavities with communicating sinuses. All × 0.7.

Optic nerve, retina, and choroid.—The optic nerve was absent, atrophied or calcified. Sometimes it ended in the sclera, and no direct connection between the eye and the optic nerve existed. In one case the optic nerve failed to develop, but an optic stalk and choroid fissure persisted. The optic stalk extended up to the rudimentary iris and remained embryonic in character, showing a developmental arrest (fig. 3, *B*).

The retina and choroid at the posterior part of the eye were normal in distribution. The retina extended over and covered the interior of the cysts.

Sclera.—The sclera around the primitive eye was thin, not well defined, and showed various defects which formed enlarged sinusus connecting with the cysts. The number of defects in the walls of the sclera depended upon the number of cysts present. Almost all the eyes showed some sort of scleral defect.

Cystic cavities.—The cystic cavities were filled with clear fluid which resembled vitreous humor. The cysts were bilocular or multilocular and the thin septum of the cysts had small defects which permitted intercommunication between the cystic cavities. The walls of the cysts were rough and uneven with various papillary projections. The walls of the cystic cavities were covered with epithelium which in some places appeared pigmented; in others pigmentation was absent.

The single cystic cavities in the older animals were 6 cm. in diameter, while the multilocular cavities were from 2 to 4 cm. in diameter. In

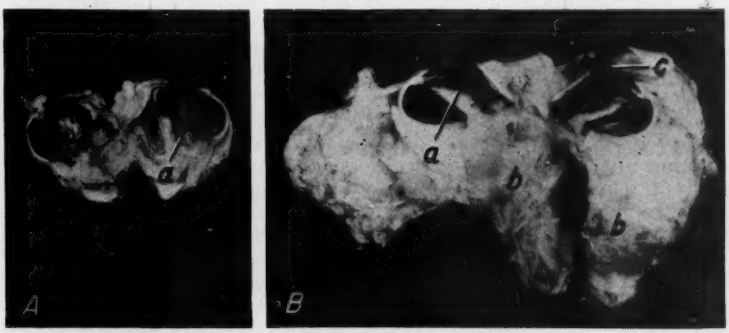

FIGURE 3.—*A*, Dumbbell-shaped lens that fills eye cavity: *a*, Lens protruding into cystic cavity. *B*, Eye showing persistence of optic stalk: *a*, Optic fissure, lens absent, indicating definite developmental arrest; *b*, large dilated cystic cavities; *c*, iris bent and fused to cornea. All × 0.7.

addition to the larger cysts, there were numerous smaller ones from 2 to 0.5 cm. in diameter, forming irregular and complex structural arrangements.

DISCUSSION

An understanding of the development of the various anomalies observed in the malformed eyes can be obtained only if we recognize the fact that embryonic growth and proliferation depend upon an unlimited supply of food and oxygen. Stockard (*13*) has pointed out that the normal development of the embyro depends upon the stability of certain factors in the environment. Embryonic death, as well as monstrous development, can be induced by changes in the conditions of moisture, temperature, oxygen supply, the action of various chemical substances, actinic rays, and dietary deficiencies. The malformations which are produced do not depend upon the specific teratogenic substance, but depend upon the time at which the developmental arrests occur. The development of a normal structure depends, not only upon a definitely located primordia, but also upon the time per-

mitted for its development. Therefore, it must attain its supremacy in growth and proliferation during a limited period. This time-limited opportunity for development is due to the growth competition between organs. Stockard (*13*) stated that if the entire embryo is depressed or has its developmental rate reduced, the rapidly developing structures will be affected more seriously than the slowly developing ones. When normal growth rate is resumed the slow-growing parts are able to regain their ordinary rates, but the rapid-growing organs are unable to resume their extraordinarily high rate of development, and, therefore, the rapidly developing organs lose their extraordinary advantages and the organ fails to develop or is malformed.

The abnormalities observed in these eyes shows developmental arrest, indicating that the anlage was there but the potential for growth and proliferation was lost. Some of the structures were in an embryonic state, which was well demonstrated by the presence of the optic stalk and the persistence of choroid fissue and embryonic lens. The different manifestations and the various grades of abnormalities indicated that injury did not occur in these animals at the same stage of embryonic development. Development was more advanced in some cases than in others, and consequently structural development was more advanced in some than in others when injury occurred. The postnatal age of the animals did not alter the defects in the structure of the eyes since the 3- and 6-months old animals had lens, iris, and corneal developments as primitive as those of a 2-week-old animal. Another interesting point observed was the fact that grossly the structures of the eyes did not show any of the degenerative changes that would be expected if the cysts exerted excessive pressure. The normal pressure in the various cavities was maintained through the sinuses and defects in the walls of the cysts and sclera. This pressure equilibrium suggests that the fluid was not foreign in origin, but was produced in the same manner as vitreous humor.

HISTOLOGICAL CHANGES IN MALFORMED EYES

OBSERVATIONS

Only a few histological studies of malformed eyes have been reported in the literature. Franke et al. (*3*) studied the effect of selenium in chick embryos but failed to give the histological or the gross changes that take place in the different organs. Warkany et al. (*14*, *17*) reported the histology of the malformations observed in the eyes of rats whose mothers were on a vitamin-A-free diet.

The eyes to be studied were fixed in 10 percent formalin, embedded in paraffin, and sections 10μ to 12μ thick were cut. The sections were stained with haematoxylin and eosin, with copper chrome haematoxylin, and with Mallory's connective tissue stain. From each eye 50 sections were taken at 100μ intervals.

Although the structural malformations of the eyes were varied and numerous, it was apparent that they were due to the failure of the various structures of the eye to fuse, lack of differentiation, lack of growth, or cellular proliferation. The failure of the sclera to fuse was the most frequent defect observed. When this happened the vitreous humor was permitted to flow into the mesoderm, where multiple cyst formation occurred.

The histological structure of the sclera indicated that there was not only an adherence of the sclera to the bony orbit, but also that endochrondral bone formation was present in the sclera. This process was not an abnormal calcification but an active bone formation. The sclera, in addition to the normal cellular structure, contained many undifferentiated mesenchymal cells.

The mesodern of the cornea failed to differentiate into its normal structures. The composition of the tissue was more like that of undif-

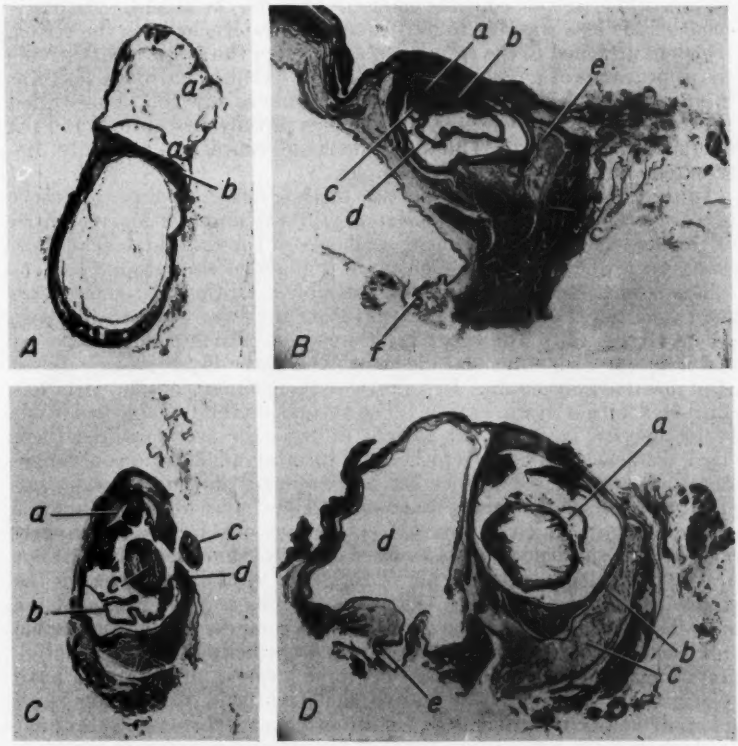

FIGURE 4.—*A*, *a*, Bilocular bulbar conjunctival cyst covered with epithelial cells; *b*, hyperplastic cornea. *B*, *a*, Pseudoanterior chamber; *b*, bent iris; *c*, ciliary processes extending over anterior part of eye; *d*, embryonic retina; *e*, embryonic tissue; *f*. papillary projection in cystic cavity. *C*, *a*. Eye shows microphthalmia with rudimentary anterior chamber; *b*. embryonic retina; *c*, lens extruded through defective sclera into cystic cavity, giving appearance of double lens because of curvature of lens; *d*, defect in sclera. *D*, *a*, Embryonic lens almost filling posterior chamber; *b*, thin sclera; *c*, embryonic tissue surrounding sclera; *d*, large lateral cyst; *e*, papillary projections on cyst shown in *d*.

ferentiated mesenchymal tissue with occasional pigmented cells and some connective tissue. The epithelium covering the cornea was normal in appearance except in a few cases in which epithelial hyperplasia was observed. In the eyes of the younger animals the bulbar conjunctiva formed systs which were covered with conjunctival epithelium (fig. 4, *A*).

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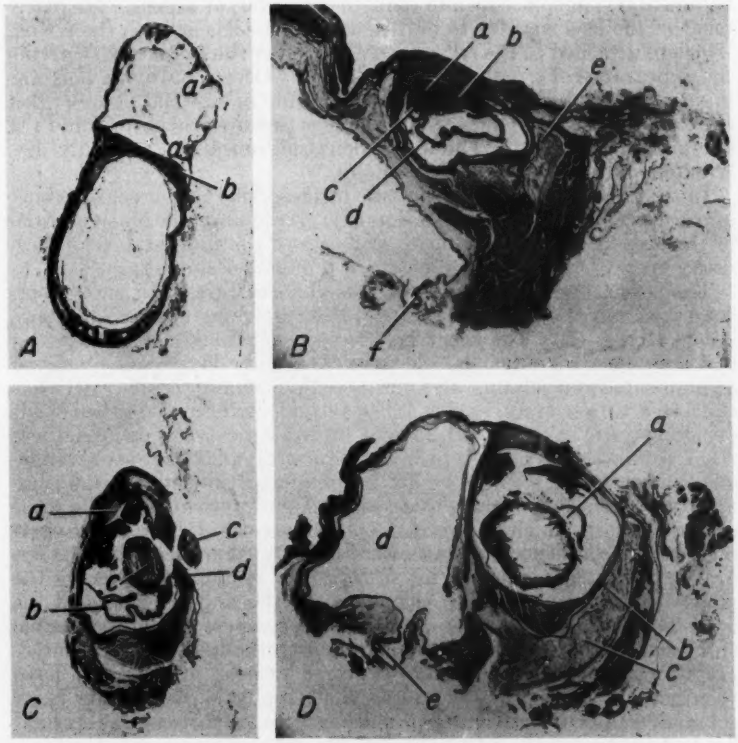

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The anterior chamber was rudimentary, shallow, or absent. The iris, at times, was absent, bent, or fused with the cornea. The bent iris formed a pseudoanterior chamber and brought about the displacement of the ciliary processes which extended over the enterior part of the eye or were attached to the cornea (fig. 4, *B*). The normal ciliary body was absent. Misplacement of the choroid occurred in one eye, and the choroid extended up to the anterior chamber where the vessels were dilated and a newly formed thrombus was present. The lens were rudimentary, misplaced, embryonic in character, or absent. Misplacement of the lens was due to its fusion with the iris or with the retina. The lens attached to the retina passed through the cystic cavity giving the appearance of a double lens (fig. 4, *C*). Occasionally the lens was highly cellular in appearance and embryonic in size and almost filled the cavity of the eye (fig. 4, *D*). The lens capsule was normal and the fibers were nucleated. The amorphous substance was unevenly distributed and there were many vacuoles.

In the abnormally everted portion of the cysts there was an absence of choriocapillary layers. Mann (*7*), discussing the abnormalities of the eye, states that the choriocapillaries of the choroid develop normally only when it is in contact with pigmented epithelium.

The retinal tissue exhibited various abnormalities. In many cases the retina revealed embryonic separation and was composed of two layers (fig. 4, *C*). The inner layer was much thicker than the outer and contained some neutral elements; the outer layer was composed of pigmented epithelium. In some cases this primary optic vesicle was converted into a cystic cavity. The retinal epithelium formed folds over the cystic cavities. At various areas in the cystic cavities there was a condensation of the epithelium, forming adenomatous arrangements (fig. 5, *A*). The core of the papillary projections was composed of nervous and connective tissue. There were areas in the walls of the cysts in which the retinal epithelium was folded and hyperplastic. The multilocular cysts were directly connected with the eye proper by multiple sinuses (fig. 5, *B*). The epithelium covering the sinuses was continuous with the cystic cavities and the retina of the eye. The walls of the cystic cavities were composed of fibrous tissue and lined with a perverse layer of retinal epithelium. Various interpretations as to the formation of perverse layering of the retina have been given. Wolff (*18*) suggested that this type of layering is an actual detachment of the retina resulting from the increased subretinal fluid which pushes the retina through the choroid fissure into the loose mesoderm. Cysts are formed in this manner by the condensation of the mesoderm outside the eye.

There were secondary cysts within the larger cysts which were covered both inside and outside with retinal epithelium. The secondary cysts formed near the optic nerve, in the retina, outside the sclera, and within the wall of the sclera or at the junction of the anterior and posterior chambers. Surrounding the cyst was a large amount of undifferentiated mesenchymal tissue. Within the cystic cavities occasionally there were structures which resembled ciliary processes containing pigmented epithelium. These were attached to the walls of the rudimentary eye and indicated that during the development of the eye some disorganization of the anlage occurred, as these structures developed outside the eye and within the cystic cavities.

The optic nerve frequently showed atrophy. In some of the eyes there was no connection between the optic nerve and the eye. In these eyes the nerve ended diffusely in the posterior part of the sclera. In two cases the optic stalk failed to develop into the optic nerve. There was also lack of differentiation in all other structures. The vitreous humor in the eye formed fine longitudinal fibrils and filled the cavity of the eye (fig. 5, *C*). The persistence of the optic stalk, the choroid fissure, and the embryonic vitreous indicated that developmental arrest occurred early in embryonic development. Mann (*7*), correlating the development of the eyes with the rest of the body, states that in the 35-mm. embryo the optic stalk is replaced by the optic nerve.

Within the eyes and surrounding the eyeball there was a great deal of undifferentiated tissue. The presence of this embryonic undifferentiated tissue indicated that development was inhibited and its

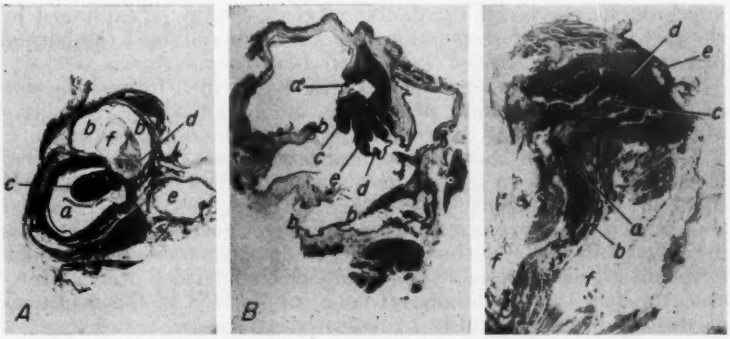

FIGURE 5.—*A*, Eye composed of two large cavities: *a*, Surrounded by sclera; *b*, surrounded by embryonic tissues; *c*, lens abnormal in shape and attached to retina; *d*, retina forming adenomatous arrangement at area of contact with lens; *e*, cyst outside eye proper lined with retinal epithelium; *f*, dilated cyst derived from retina. *B*, *a*, Defect in wall of sclera; *b*, intercommunicating sinuses lined with retinal epithelium; *c*, ciliary process attached near scleral defect at posterior part of rudimentary eye; *d*, dilated secondary cyst; *e*, retinal tissue forming adenomatous arrangement with dilated secondary cyst shown in *d*. *C*, *a*, Optic stalk; *b*, choroid fissure; *c*, filamentous vitreous; *d*, cornea, which appears homogeneous; *e*, single layer of epithelium covering cornea; *f*, muscle bundles disorganized and separated by large cyst; *fo*, muscle bundle.

potential for differentiation was lost. In contrast to this undifferentiated tissue were the tissues which maintained their developmental potential and differentiated at abnormal positions.

DISCUSSION

The malformed eyes investigated presented a bizarre histological picture. The malarrangements of these structures may be explained as a result of early embryological arrest and disturbance in the anlage. The factors which might have caused these changes are numerous. The developmental arrest may have been due to decreased oxygen caused by the action of selenium. The lack of growth and cellular proliferation at certain developmental stages may have been responsible for the rudimentary and malformed structural development. The decrease of vitamin A due to selenium may also have been a

contributing factor to these malformations. The writers (*11*) have observed a decrease in vitamin A and ascorbic acid in selenium poisoning, and Warkany (*14*) has described malformation of the eye with vitamin A deficiency where the vitreous was fibrous in character.

Normally ruminants can synthesize ascorbic acid, and the embryo is able to supply the necessary amount of ascorbic acid for its development. The decrease in ascorbic acid during embryonic development may have caused certain of the defects. The normal function of ascorbic acid is to maintain the integrity of the intercellular substance of mesenchymal supporting tissues. Only in one case were there definite signs of vascular damage suggesting avitaminosis, although the failure of the different mesenchymal structures to maintain their continuity during embryonic development may have been due to vitamin C deficiency.

The fact that the fluid present in the multiple cysts did not produce any gross or microscopic pathological changes may be explained by the fact that the intercommunicating sinuses maintained the normal pressure within the eyes.

The neural tissue within the cystic cavities, which formed the core of the numerous papillary projections, indicates that the epithelium of the retina served as internal covering for the cystic cavities.

The derivation of the endochondral bone from the sclera observed in some cases poses an interesting question. In the lower vertebrates the sclera is cartilagenous. Whether the presence of endochondral bone within the sclera in mammals indicates a reversion under stress to the less differentiated tissue or whether the bone formation was due to misplaced mesenchymal tissue cannot be stated definitely. However, the fact that endochondral bone was formed in the sclera indicates the pluripotentiality of mesenchymal tissue.

SUMMARY

A description of the gross malformations of the eyes observed in the progeny of ewes grazed on seleniferous areas is presented. Congenital microphthalmia, rudimentary eyes, displacement of the lens, absence of the lens, lack or normal division between the cornea and sclera, microcornea, and colobomas of the various structures are described.

Histological studies indicated that in the malformed eyes cellular and structural misplacement occurred during early embryonic development. The majority of the defects involved the structures which developed from mesenchyme. Endochondral bone formation within the sclera indicates the pluripotentiality of the mesenchymal tissue. Histological changes in the various structures of the eyes are discussed.

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REACTION OF MOUNTAIN BROME AND CANADA WILD-RYE STRAINS TO HEAD SMUT (USTILAGO BULLATA)[1]

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INTRODUCTION

In the Pacific Northwest and the Rocky Mountain States several species of forage grasses are commonly found affected with head smut (*Ustilago bullata* Berk.) in native stands and in plantings for hay or pasture, seed production, or soil and water conservation. Prominent among these species is mountain brome (*Bromus marginatus* Nees), a native perennial. This grass is one of the best forage species for use with sweetclover in the intermountain area. As part of a general program for improvement of the species, attention has been given to head smut resistance. This paper presents the reaction of several agronomically superior accessions of mountain brome to various races of head smut in controlled inoculation tests. *Elymus canadensis* L., another native species which is susceptible to the same races of head smut, has also been tested and the results are included herein.

MATERIALS AND METHODS

Fourteen accessions of mountain brome selected from field collections representing widely varying types from several Western States have been tested for degree of susceptibility to 4 races of head smut attacking this species. Two of these (race 5 and race 7) are among those described earlier,[2] and 2 (race 9 and race 12) are new races that have since been differentiated. The inoculations were performed by the partial-vacuum method. The inoculated seed was germinated in plant bands in the greenhouse in late winter, and after the seedlings were well above the soil they were moved outside for vernalization. The vernalized seedlings were transplanted to nursery rows in the spring, and most of them headed well the following summer. The same procedure was used for 12 accessions of *Elymus canadensis*.

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TABLE 1.—*Reaction of accessions of Bromus marginatus and Elymus canadensis to 12 collections of head smut from Bromus spp., and comparative virulence of the races*

Species inoculated and accession No.[1]	Smut (head-count basis)[2] caused by indicated race and collections from different species of *Bromus*												Collections infecting	Average smut	Susceptibility index
	Race 5			Race 7						Race 9		Race 12			
	B. marginatus		*B. anomalus*	*B. catharticus*				*Bromus* sp.	*B. inermis*	*B. marginatus*		*B. purgans*			
	M–C	M–W	M–L	M–I	M–I_1	M–I_2	M–R	M–T	M–Y	M–C_1	M–C_2	M–V			
Bromus marginatus:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	
Wn–439	0	0	0	0.7	0.3	0	0	0	0	0	0	0	2	0.1	0.2
Wn–438	16.3	0	0	0	0	0	0	0	62.0	0	62.4	21.8	4	13.5	54
P–1841	94.6	95.4	97.9	63.1	47.4	67.0	70.7	4.0	49.2	90.1	83.5	92.9	12	71.3	856
P–2133	6.4	0	0	0	0	0	0	0	2.0	0	19.1	15.1	4	3.6	14
P–3972	83.1	58.6	49.2	28.9	38.2	42.4	44.1	16.0	52.2	100	88.0	86.9	12	57.3	688
P–3368	73.2	32.4	70.0	9.6	25.9	39.1	23.0	1.5	52.0	4.0	57.5	71.2	12	38.3	460
P–5355	87.3	0	0	0	.3	0	0	0	61.1	84.6	40.1	57.5	6	27.6	166
P–5391	24.9	68.8	2.9	6.0	5.1	1.2	3.7	8.8	46.4	21.8	84.4	79.1	12	29.4	353
P–5722	87.0	91.1	1.4	75.6	67.5	65.4	65.3	19.4	93.0	85.3	64.5	89.5	12	67.1	805
P–6268	10.5	86.2	6.9	0	0	0	0	1.8	53.4	43.1	27.6	67.4	8	24.5	196
P–6328	12.2	5.9	97.7	46.1	36.5	.7	0	1.3	2.4	27.6	10.7	76.1	11	26.4	290
P–7255	16.0	83.1	.3	.4	.4	0	0	3.8	1.2	3.4	.2	73.4	10	15.2	152
P–4824	91.2	0	1.9	0	0	23.4	0	.9	2.0	1.2	77.7	94.3	8	24.4	195
P–2725	43.3	93.8	81.6	0	0	0	0	0	0	100	65.7	13.2	6	33.1	199
Elymus canadensis:															
F–9	16.5	11.8	60.4	39.8	10.1	30.1	14.4	2.1	6.8	.4	24.5	17.3	12	19.5	234
P–786	28.8	66.5	61.6	80.6	73.6	59.6	30.6	23.5	32.9	[3]	42.4	57.8	11	50.7	558
P–788	50.8	86.7	89.1	88.1	70.0	95.3	90.5	93.2	64.9	3.2	84.2	88.0	12	75.3	904
P–2389	71.1	35.4	68.2	93.6	97.6	99.4	84.6	85.5	50.9	21.3	88.4	82.9	12	73.2	878
P–2623	45.8	78.6	84.3	96.1	81.9	78.8	86.3	63.9	36.6	2.3	36.6	57.0	12	62.4	749
P–2624	42.9	74.8	81.9	93.1	42.3	66.9	81.1	64.3	26.1	3.5	34.2	47.3	12	54.9	659
P–3355	0	72.0	15.9	53.4	20.2	49.5	41.2	6.2	0	0	0	48.9	8	25.6	205
P–4597	36.3	30.1	75.7	86.9	86.6	77.6	83.2	64.6	35.4	0	13.3	63.4	11	54.4	598
P–4659	4.6	5.7	38.5	77.3	52.5	87.1	63.3	20.2	11.9	5.7	16.6	10.9	12	32.6	391
P–4669	0	54.9	15.9	4.8	6.4	0	6.6	24.2	.2	0	5.2	20.0	9	11.5	104
P–4678	20.3	10.8	3.6	36.8	21.2	27.8	29.5	2.5	1.4	5.4	2.0	4.4	12	13.8	166
P–4681	0	47.6	0	30.8	26.8	27.0	17.7	10.2	31.8	28.9	1.4	1.2	10	18.6	186
Accessions smutted	22	21	21	20	21	18	17	21	23	19	24	25			
Average percent smut	37.0	45.8	38.7	38.9	31.2	36.1	32.0	19.9	29.7	25.3	40.0	51.4			
Virulence index	814	962	813	778	655	650	544	418	683	481	960	1,285			

[1] Wn numbers are those of the Washington Agricultural Experiment Station; P numbers, those of the Division of Nurseries, Soil Conservation Service; and the F number, that of the junior author.

[2] In most cases the number of heads per row ranged from 300 to 600 for *B. marginatus* and from 400 to 700 for *E. canadensis*.

[3] No stand.

RESULTS

In 1941, 14 accessions of mountain brome were each inoculated with 12 collections of head smut from widely separated localities in the Western States, representing 4 physiologic races. At the same time 12 accessions of Canada wild-rye were inoculated with the same smut collections. The purpose of the experiment was twofold: (1) To obtain a comparative evaluation of the resistance of the mountain brome and Canada wild-rye accessions previously determined as being agronomically superior and (2) to obtain a comparison of the virulence of the 4 races of head smut and of the collections which comprise these races. Data were taken in 1942 on a head-count basis, and the results are shown in table 1.

The data from these inoculations present some interesting results. In the first place, the differences between accessions in susceptibility to head smut are very great indeed. No 2 accessions of mountain brome gave the same reaction, the range of infection being 0 to 100 percent. Only 2 accessions appeared resistant to all 12 collections of smut; one (Wn–439) was practically immune, while the other (P–2133) appeared generally resistant or immune. Although several accessions of mountain brome were more or less infected by all 12 of the head smut collections, none of the accessions was definitely susceptible to all of them. The nearest approach to this was P–3972, for which the lowest percentage of smut was 16, obtained with collection M–T (race 7), and the highest was 100, obtained with collection M–C_1 (race 9).

A ready evaluation of the comparative resistance of the 14 accessions of mountain brome is provided in the susceptibility index in table 1. Neither the average percentage of smut nor the number of collections infecting is by itself a satisfactory index of susceptibility. The susceptibility index, the product of the average percentage of smut in a given accession and the number of collections infecting that accession, is considered a much better measure. By this measurement the outstanding immunity of Wn–439 is readily apparent as this accession has a susceptibility index of only 0.2. Other accessions also exhibit a high degree of resistance to most of the collections (Wn–438 and P–2133 with susceptibility indexes of 54 and 14, respectively), but they are more or less susceptible to 1 or 2 collections. Accession P–2133 (a reproduction of Wn–438) represents an early-maturing strain of mountain brome that has been in commercial production for about 10 years in Washington and probably elsewhere in the Northwest. Fortunately, it appears to be resistant.

Very much the same results were obtained with the 12 accessions of Canada wild-rye, except that the general susceptibility was even higher than with the brome accessions. The lowest susceptibility index was 104 and the highest was 904 (out of a theoretically possible 1,200). Accessions P–788 and P–2389 both were high in susceptibility.

Considerable differences in pathogenicity were observed in the 12 head smut collections used in the inoculations summarized in table 1. The disturbing fact came to light that all of the 12 collections seem to be different even though only 4 races are represented. Theoretically, it might be expected that all collections previously identified as belonging to 1 race should have given the same reaction on the 26

Bromus and *Elymus* accessions inoculated, but such certainly was not the case. The best instance for analysis is seen in collections M–I, M–I_1, M–I_2, M–R, M–T, and M–Y, all of which belong to race 7, on the basis of the reaction of the head smut differential grasses to these collections. However, on the basis of the reactions of P–1841, P–5355, P–6268, P–6328, P–2624, and P–4824 these 6 smut collections are all different. Similarly, the 3 collections belonging to race 5 and the 2 belonging to race 9 are easily differentiated on the basis of the reaction of some of the 26 host accessions used.

Further comparison of the 12 smut collections is possible by means of the virulence index shown at the bottom of table 1. The virulence index is the product of the average percentage of smut produced by a collection or race and the number of accessions of host plants which it was capable of infecting. Here, again, the collections within a race seem to differ. Thus, according to this index, collection M–C_2 is twice as virulent as collection M–C_1, and yet they are considered as belonging to the same physiologic race. However, it is not yet considered feasible to attempt to differentiate races by means of differences in virulence index. Rather it seems sufficient to recognize that the physiologic races previously described in *Ustilago bullata* are not genetic entities. The data in table 1 suggest that at least some of these races are composed of biotypes that can be separated by the use of additional hosts or strains within a host species.

The experiment just described yielded one result of a discrepant nature. Accession P–3368 was infected by all 12 collections, with an average of 38.3 percent of smut, and a susceptibility index of 460 (table 1). In previous preliminary tests this accession had exhibited a high degree of resistance and in the observational rows of the Soil Conservation Nursery at Pullman, Wash., it had remained smut-free for several years. The sudden susceptible reaction of P–3368 in the present experiment was, therefore, unaccountable. It was decided to conduct further inoculation experiments involving P–3368 and 5 other accessions of mountain brome of excellent agronomic qualities.

In the second experiment six accessions of *Bromus marginatus* were each inoculated with four races of head smut. In this test, inoculations were made with mixtures of collections of each race rather than with individual collections, because the primary interest was in testing the grass accessions for resistance. In the hope of finding in the susceptible accessions smut-resistant individuals that were agronomically desirable, the experiment was conducted and data were taken on a spaced-plant basis. The inoculated seed was planted in the greenhouse, and the seedlings were transplanted to individual, small, wood-veneer plant bands. A period of vernalization preceded the transplanting to the field in the spring of 1943. Most of the plants headed well in 1943, and reaction data were taken in 1944. These data are summarized in table 2.

The results of the second experiment (table 2) further indicate the apparent immunity of Wn–439 to head smut. This experiment reestablished the high degree of resistance in P–3368 and made the susceptible reaction of this accession in the first experiment even more subject to question. The high degree of susceptibility of P–3972 and P–5355 (susceptibility index 371 and 359, respectively) again is well

demonstrated. However, in no case were 100 percent of the plants infected with smut. The smut-free plants represent instances either of escape from infection or of genetic resistance. This investigation is being continued.

It will be noted that in this second experiment accession P–2133 appeared definitely susceptible to race 7 and race 12, a reaction quite in contrast to the general resistance manifest in the first test. In this connection it should be pointed out that preliminary inoculations in previous years had indicated that P–2133 is highly resistant to most collections of head smut but more or less susceptible to others.

The reactions of the six accessions of mountain brome shown in table 2 do not reveal any great differences in the general virulence of the four races of head smut. All have nearly the same virulence index.

TABLE 2.—*Reaction of 6 accessions of Bromus marginatus to 4 races of Ustilago bullata, and comparative virulence of the races*

Accession No.[1]	Race 5		Race 7		Race 9		Race 12		Races infecting	Average smut	Susceptibility index
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected			
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Per cent*	
Wn–439[2]	34	0	15	0	13	0	13	0	0	0	0
P–2133	101	3.0	73	37.0	84	17.9	94	53.2	4	27.8	111
P–3368	101	3.0	126	6.4	124	.8	72	0	3	2.5	8
P–3972	97	99.0	92	84.8	79	98.7	54	88.9	4	92.8	371
P–5355	96	99.0	94	98.9	56	94.6	21	66.7	4	89.8	359
P–5391	111	50.5	129	24.8	104	63.5	123	98.4	4	59.3	237
Accessions smutted	5		5		5		4				
Average percent smut	42.4		42.0		45.9		51.2				
Virulence index	212		210		230		205				

[1] See footnote 1, table 1.
[2] There was insufficient seed of this accession to provide the desired minimum of 100 plants per race of smut.

The discrepant results obtained with accession P–3368 of mountain brome, as described, suggested error either in the identity of the seed used in the first experiment (table 1) or in the smut collections used as inoculum. The possibility of an error in the identity of the seed used seemed under the circumstances the more plausible, and it was decided to investigate that possibility first. Seed of P–3368 produced in 5 different years was used: 1936, 1938, 1940, 1941, and 1942. The 1940 seed was from the same lot that had been used in the first inoculation experiment, summarized in table 1, in which P–3368 was so generally susceptible. These five seed lots were each inoculated with six collections of head smut, five of which were the ones which produced the highest percentages of smut on P–3368 in the first experiment. The seed was inoculated and planted as in the other experiments. The 1936 seed was no longer viable, and no stands were obtained. The data taken on a head-count basis are presented in table 3.

TABLE 3.—*Comparative susceptibility of 4 seed lots of Bromus marginatus P–3368 produced in different years to various races and collections of Ustilago bullata*

Year seed was produced	Smut (head-count basis) produced by indicated race and collection						Average
	Race 5		Race 7 (M–Y)	Race 9 ($M–C_2$)	Race 12		
	M–C	M–L			M–V	M–Z	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1938	0	10.3	4.3	0	0	0	2.4
1940	35.4	40.3	62.5	74.1	84.2	60.3	59.5
1941	0	.5	.5	4.9	2.4	2.2	1.8
1942	0	0	0	0	.9	0	.2

A comparison of the results with the four seed lots of P–3368 in table 3 indicates that the 1940 lot, which gave the discrepant results in the first experiment, was not accession P–3368, as labeled, but probably represents some susceptible selection of *Bromus marginatus* or perhaps another accession of the same grass.

DISCUSSION

From the foregoing results it appears that within the species *Bromus marginatus* there are strains varying markedly in their reaction to races or collections of head smut. Two types of mountain brome of economic importance are easily distinguished by their growth habits. The first, represented by Wn–438 and P–2133, is an early-maturing, fairly leafy strain and the second, represented by Wn–439 and P–3368, is a late-maturing, very leafy strain. Both types are satisfactory with regard to seed yield, but the latter type by virtue of its lateness and leafiness is the more desirable strain for use with sweetclover.

Before accessions or selections are released for increase or commercial production, reaction to head smut should be determined; and since resistant strains are available, preference should be given to these. While head smut can be controlled by proper seed treatment, the use of resistant or immune strains eliminates the necessity of this operation. In the present investigations, two accessions of mountain brome were outstanding for their resistance to head smut: Wn–439 and P–3368. The first is a Washington Agricultural Experiment Station selection; the second is an increase of Wn–439 made by the Soil Conservation Service at Pullman, Wash. Further study of strains of mountain brome is being made with the intention of releasing the one that proves most resistant to smut and at the same time is superior for use as forage.

SUMMARY

Fourteen accessions of mountain brome (*Bromus marginatus*) and 12 of Canada wild-rye (*Elymus canadensis*) have been tested for susceptibility to races of head smut (*Ustilago bullata*) known to attack mountain brome. Considerable differences in degree of susceptibility were found in the mountain brome accessions, ranging from high resistance to high susceptibility. The same was true for Canada wild-rye, but this species was more generally susceptible than mountain brome.

Since some lines of mountain brome are highly susceptible to head smut, it is essential that the reaction to this smut be determined before strains of the grass are increased for seed or released for commercial production.

It appears probable that none of the physiologic races of *Ustilago bullata* that have been differentiated and described represents a genotypic entity, but rather that they are phenotypes whose component parts may be separated by the use of additional differential hosts or strains within a host species.

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